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Efficiency of energy transfer from photosystem II to photosystem I in *Porphyridium cruentum*

(energy distribution/chlorophyll/phycobilin/phycoerythrin/photosynthetic apparatus)

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ABSTRACT **The yield of energy transfer from photosystem II to photosystem I in *Porphyridium cruentum* varies from a minimum value of about 0.50 when the photosystem II reaction centers are all open to a maximum value between 0.90 and 0.95 when the centers are all closed.**

Studies of the effects of different quenching agents on light-induced fluorescence yield changes in chloroplasts at −196° (1, 2) led to a model for the photochemical apparatus of photosynthesis (3), which has been used to determine the distribution of excitation energy between photosystem I (PSI) and photosystem II (PSII) (4, 5). Two types of parameters were incorporated into the equations of the model to describe the distribution of energy. The initial distribution is specified in terms of α, the fraction of the absorbed quanta delivered initially to PSI, and β, the fraction delivered to PSII, where $\alpha + \beta = 1$. Following the initial distribution some of the quanta in β fraction going to PSII may be transferred to PSI. A photochemical rate constant term, $k_{T(II\rightarrow I)}$, was introduced into the equations to account for such a process and the yield of energy transfer from PSII to PSI, $\varphi_{T(II\rightarrow I)}$, was shown to depend on the state of the PSII reaction centers in the same manner as the yield of fluorescence from PSII [i.e., the yield increases from a minimum level, $\varphi_{T(II\rightarrow I)(o)}$, when the PSII reaction centers are all open ($A = 1$) to a maximum level, $\varphi_{T(II\rightarrow I)(M)}$, when the centers are all closed ($A = 0$)].

The value of α for spinach chloroplasts was estimated to be about 0.3 for blue light excitation from studies of fluorescence at −196° in the presence and absence of Mg^{2+} (4). Furthermore, it was shown that α was practically independent of the wavelength of excitation from 400 to 680 nm, although α became unity between 680 and 700 nm as PSI became the dominant absorber (5). The invariance of α over the major part of the visible spectrum was attributed to the accessory pigment role of the light-harvesting chlorophyll *a/b* protein and to the fact that the spectral characteristics of the chlorophyll *a/b* protein are similar to those of the chlorophyll in PSI and PSII. Recently, the wavelength dependence of α was determined for a red alga, *Porphyridium cruentum*, (6) in which the absorption spectra of the light-harvesting phycobilin accessory pigments are very different from the spectrum of the chlorophyll. In this case, it was found that α was close to unity at wavelengths absorbed primarily by the chlorophyll, both in the blue between 400 and 450 nm and in the deep red longer than 680 nm, while α was close to zero in the 550-nm region absorbed by phycoerythrin. These results indicate that almost all of the chlorophyll in *Porphyridium* is associated with PSI but that the phycobilisomes transfer excitation energy almost exclusively to the small amount of chlorophyll that is associated with PSII. The action spectrum for the photosynthetic O_2 evolution of these cells shows very little activity in the blue and deep red regions where $\alpha = 1$ because of the absence of PSII excitation. However, the action spectrum follows the absorption spectrum of the phycobilisomes fairly closely, showing a peak in the 550 nm region. At these wavelengths, where α is close to zero, there must be considerable energy transfer from PSII to PSI in order to drive the entire linear electron transport chain. The purpose of the work reported here was to estimate the yield of energy transfer from PSII to PSI in *Porphyridium* cells, especially at the minimum level when the PSII reaction centers are all open.

Abbreviations: PSI, photosystem I; PSII, photosystem II.

MATERIALS AND METHODS

Unialgal cultures of the red alga *Porphyridium cruentum* were grown in an artificial sea water medium (7) at 19° in 125 ml flasks on a rotary shaker with diffuse lateral illumination (80 $\mu W/cm^2$) from fluorescent lamps. After 7–10 days of growth the cells were collected by centrifugation at 5000 × *g* for 5 min and resuspended in fresh growth medium. All measurements of fluorescence and absorbance were carried out in 0.5 ml suspensions of cells frozen to −196° in our vertical cuvette and Dewar system (8).

Absorption and fluorescence excitation spectra were measured with our computer-linked, single-beam spectrophotometer as described previously (5). The fluorescence excitation spectra were corrected for the quantum flux of the excitation light from the scanning monochromator. The exciting light was incident on the top surface of the frozen samples and fluorescence at 722 nm (defined by a filter combination consisting of a Toshiba VR-65 glass filter, a Corning 9830 glass filter, a Corning 5031 glass filter, and a 723 nm Balzers interference filter) was measured from the bottom surface.

Fluorescence emission spectra were measured from the top surface of the frozen samples as described previously (9) with a Bausch and Lomb high intensity monochromator and a Ga-As phototube (Hamamatsu R666S). The measurements were taken on line with a small computer so that difference spectra could be computed and plotted. The curves presented are direct recordings from the computer.

RESULTS

Given the conclusions stated in the *introduction* that α is close to unity for light absorbed by the chlorophyll of *Porphyridium* cells and is close to zero for light absorbed by the phycoerythrin, it appeared that it might be possible to estimate the yield of energy transfer from PSII to PSI from a comparison of the fluorescence excitation spectrum of PSI fluorescence with the absorption spectrum of the cells. The fluorescence excitation spectrum, corrected for the incident quantum flux, was measured for 722 nm fluorescence at the F_M level at −196° for a very dilute suspension of cells (see middle curve in Fig. 1). The concentration of cells was much too low for absorbance measurements, so that the absorption spectrum presented in Fig.

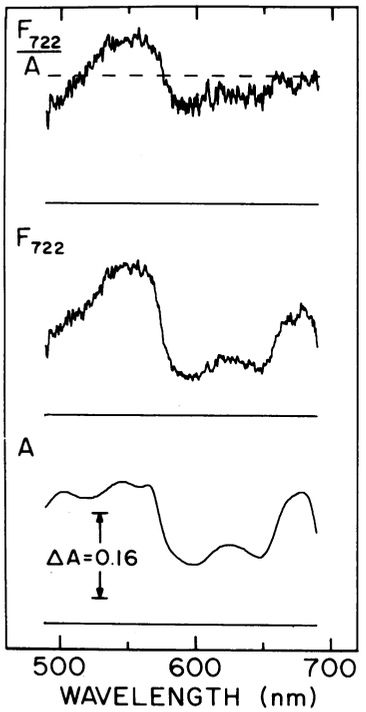

FIG. 1. Lower curve: absorption spectrum of *Porphyridium* cells at −196°. Middle curve: fluorescence excitation spectrum for fluorescence at 722 nm corrected for equal quantum flux. The cell suspension was diluted 100-fold from that used for the absorption spectrum, frozen to −196°, and preirradiated with white light to establish the F_M level. Upper curve: the ratio of the fluorescence excitation spectrum to the absorption spectrum. The broken line indicates the relative value of the ratio at 685 nm.

1 was measured at −196° on a sample of cells 100 times more concentrated. At the very low concentrations used for the fluorescence measurements, no appreciable errors are introduced by comparing the fluorescence excitation spectrum, measured on a linear photometric scale, with the absorption spectrum measured on a logarithmic scale. If the fluorescence measured at 722 nm is due entirely to emission from PSI, the ratio of the intensity of fluorescence excited at 545 nm, $(F_{722})_{545}$, to the intensity excited at 685 nm, $(F_{722})_{685}$, divided by the ratio of the absorbances at 545 and 685 nm, A_{545}/A_{685}, should be equal to the yield of energy transfer from PSII to PSI. Or stated in an equivalent form:

$$\frac{(F_{722})_{545}}{A_{545}} = \varphi_{T(II\rightarrow I)(M)} \frac{(F_{722})_{685}}{A_{685}}$$

When the fluorescence excitation spectrum was divided by the absorption spectrum (see upper curve in Fig. 1), we obtained the untenable result that the ratio of the fluorescence excitation to the absorbance at 545 nm was 30% greater than at 685 nm. This, of course, led to a reexamination of our initial assumptions and to the suspicion that a part of the fluorescence measured through our 722 nm filter system was due to PSII emission when the excitation was at 545 nm. If excitation at 685 nm excited only PSI emission while excitation at 545 nm excited both PSI and PSII emission, our estimation of the yield of energy transfer would be in error in the direction indicated.

Fluorescence emission spectra from a dilute suspension of cells of *Porphyridium cruentum* frozen to −196° are presented in Fig. 2. Fig. 2A shows the results obtained with 545 nm excitation light absorbed primarily by phycoerythrin. The dominant emission band at 694 nm is ascribed to the antenna chlorophyll in PSII and the band at 712 nm to the antenna chlorophyll in PSI. The emission bands at 642 and 662 are due to phycocyanin and allophycocyanin, respectively. We attribute the shoulder at 680 nm to allophycocyanin B, discovered recently by Glazer and Bryant (10), which has an emission band at −196° at 680 nm. The small band at 750 nm can be attributed to PSII chlorophyll on the basis of data in the rest of Fig. 2.

Fig. 2B shows the results obtained when the same sample of frozen cells was excited with 445 nm light absorbed primarily by chlorophyll. The fluorescence spectrum is dominated by a single emission band at 712 nm, which we attribute to fluorescence from PSI chlorophyll. Fluorescence from *Porphyridium* cells excited by such wavelengths in the blue shows no fluorescence of variable yield (6). We take this fluorescence spectrum to represent pure emission from PSI chlorophyll.

The fluorescence spectrum in Fig. 2A is due to emission from both PSII and PSI while that in Fig. 2B is assumed to be due solely to PSI. Presumably, it should be possible to approximate

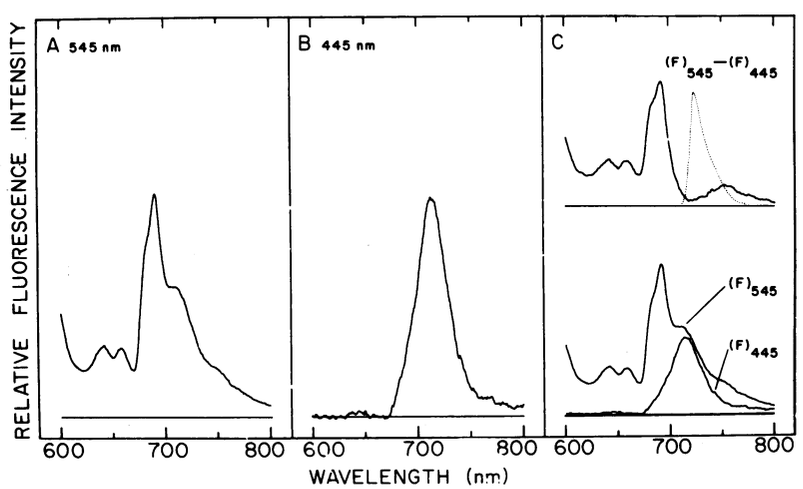

FIG. 2. (A) Fluorescence emission spectrum of a suspension of *Porphyridium* cells frozen to −196° ($A_{440} \simeq 0.05$) excited with 545 nm light at the F_M level. (B) Fluorescence spectrum from the same sample excited at 445 nm. (C) Same spectra adjusted in intensity so that the difference spectrum $(F)_{545} - (F)_{445}$ would not have any of the 712 nm emission band of $(F)_{445}$. The dotted curve is the transmission spectrum of the 722 nm filter combination used to define the fluorescence band for the fluorescence excitation spectrum in Fig. 1.

a pure PSII fluorescence spectrum by subtracting the PSI component from the PSII plus PSI spectrum. Various difference spectra (spectrum excited at 545 nm) $- k$ (spectrum excited at 445 nm) were computed with the value of k being varied until there was no 712 nm component left in the difference spectrum. Our best estimation of a pure PSII emission spectrum is shown as the difference spectrum in Fig. 2C. The degree of overlap between the 722 nm filter system which defined our fluorescence measurement and the PSI and the PSII emission spectra were calculated by graphical integration. (The transmission spectrum of the 722 nm filter combination is shown as the dotted curve in Fig. 2C.) This calculation indicated that about 25% of the 722 nm fluorescence measured with 545 nm excitation was due to PSII. If we apply this correction to our previous calculation of the yield of energy transfer [i.e., divide the ratio $(F_{722})_{545}/A_{545}$ by 1.25] we obtain that $\varphi_{T(II\rightarrow I)(M)} = 1.04$. This corrected value is still too high because it is based on the assumption that $\alpha = 0$, whereas, in fact, α is only close to zero. Our measured value of α at 545 nm was 0.05 ± 0.02 but the errors inherent in the measurement, mainly due to the overlap of PSI and PSII emission bands, would tend to decrease the measured value of α. We would estimate that the true value of α at 545 nm might be as large as 0.10 as an upper limit. To the extent that there is direct excitation of PSI by 545 nm light, there is less energy transfer from PSII to PSI. Taking these various factors into account, a conservative estimate of $\varphi_{T(II\rightarrow I)(M)}$ can be taken as 0.90–0.95. Such a value is reasonable, since the yield of energy transfer must be less than unity by at least the yield of PSII fluorescence at −196°; and a reasonable estimate for the latter is 0.05–0.10 at the F_M level (11).

It is apparent from the equations of our model that the ratio of the yields of energy transfer at the maximum and minimum levels should be equal to the ratio of the yields of PSII fluorescence at the maximum and minimum levels. The latter ratio can be measured quite readily; with these *Porphyridium* cells at −196°, F_M/F_o at 693 nm is 1.85 ± 0.05. Thus, we conclude that $\varphi_{T(II\rightarrow I)(o)}$ is close to 0.50.

DISCUSSION

The photosynthetic apparatus of *Porphyridium*, which seemingly evolved at regions in the sea where there was relatively little light at wavelengths absorbed by chlorophyll, is well suited to use the light in the middle part of the visible spectrum, which could reach those regions. The phycobilisome accessory pigment system absorbs that light efficiently and transfers most of the excitation energy to PSII. From there, the distribution between PSI and PSII is determined by energy transfer. Our conclusion that the yield of energy transfer from PSII to PSI is about 0.5 when the PSII reaction centers are all open indicates that the photochemical apparatus of *Porphyridium* has been optimized for the excitation energy from the accessory pigment system. Two of the prerequisite conditions for maximal photochemical efficiency, i.e., open PSII reaction centers and an equal distribution of energy between PSI and PSII, tend to be maintained automatically. If the PSII reaction centers start to close, more energy is transferred to PSI, which acts to reopen the closed PSII centers and restore the equal distribution of energy.

It was apparent from our previous study of the effects of Mg^{2+} on the distribution of excitation energy in spinach chloroplasts (4) that both the initial distribution, as indicated by α, and the subsequent redistribution by energy transfer from PSII to PSI determined and controlled the partitioning of energy between PSI and PSII. However, under conditions where most of the PSII reaction centers were maintained in an open state, α played a more important role than the yield of energy transfer. In our current studies of *Porphyridium*, where light absorbed by PSI appears to be relatively unimportant for photosynthesis, energy transfer from PSII to PSI plays the dominant role in determining the distribution of energy that is used for photosynthesis. Such differences between green plants and red algae are clearly related to the structural organization of the photochemical apparatus.

Although there are significant structural and organizational differences between the photochemical apparatus of *Porphyridium* and that of green plants, the model we developed from measurements on spinach chloroplasts is sufficiently general to accommodate the results we report here on *Porphyridium*. The equations derived from the model (3, 4) for the yield of PSII fluorescence and the yield of energy transfer from PSII to PSI are:

$$\varphi_{FII} = \frac{\beta k_{FII}}{k_{FII} + k_{DII} + k_{T(II\rightarrow I)} + k_{TII}} \left(A + \frac{1 - A}{1 - \varphi_{TII}\varphi_{tII}}\right)$$

$$\varphi_{T(II\rightarrow I)} = \frac{k_{T(II\rightarrow I)}}{k_{FII} + k_{DII} + k_{T(II\rightarrow I)} + k_{TII}} \left(A + \frac{1 - A}{1 - \varphi_{TII}\varphi_{tII}}\right)$$

where the ks are rate constants for the utilization of excitation energy in the antenna chlorophyll of PSII by fluorescence, k_{FII}; by nonradiative decay processes, k_{DII}; by energy transfer from PSII to PSI, $k_{T(II\rightarrow I)}$; and by trapping by the PSII reaction centers, k_{TII}. The factor in parentheses specifies that the yield of fluorescence increases from a minimum, F_o, level when $A = 1$ to a maximum, F_M, level when $A = 0$ and that the percentage increase is determined by the product $\varphi_{TII}\varphi_{tII}$, where φ_{TII} is the yield of trapping by the PSII reaction centers ($\varphi_{TII} = k_{TII}/\Sigma k_{II}$) and φ_{tII} is the probability that an exciton trapped by a closed PSII reaction center will be transferred back to the antenna chlorophyll of PSII. If there are no nonradiative decay processes acting at the reaction center chlorophyll and if we do not assume any energy transfer from the PSII reaction centers to PSI, φ_{tII} is assumed to be unity. We can accommodate the results obtained from *Porphyridium* at −196° with 545 nm excitation quite nicely if, for simplicity, we take k_{DII} to be negligible and φ_{tII} to be unity and assume that k_{FII}, $k_{T(II\rightarrow I)}$, and k_{TII} are in a ratio of 1:10:9, respectively. For such a ratio $\varphi_{TII} = 9/20 = 0.45$, which indicates $(F_M/F_o)_{693}$ should be 1.82. Our experimental value was 1.85 ± 0.05. Furthermore, the yield of energy transfer from PSII to PSI would increase from a value of 0.50 when $A = 1$ to a value of 0.91 when $A = 0$, again within the limits we predict from experimental results. The relatively low value for the ratio $(F_M/F_o)_{693}$ for *Porphyridium* of less than 2, compared to values of 5 or more obtained with spinach chloroplasts, is due to the greater degree of energy transfer from PSII to PSI in *Porphyridium* cells. It is apparent from the equations that k_{DII} processes decreas the ratio of F_M/F_o for PSII fluorescence and, so far as PSII is concerned, $k_{T(II\rightarrow I)}$ is one type of nonradiative decay process.

We take the results obtained from *Porphyridium* and our analysis of these results to represent a further confirmation of the general validity of our model for the photochemical apparatus of photosynthesis. An important conclusion from the results reported here is that energy transfer from PSII to PSI plays a major role in determining the distribution of excitation energy in the photochemical apparatus of this red alga.

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